

Services Provided in Support of the Planetary Quarantine Requirements
 of the
 National Aeronautics and Space Administration
 under Contract W-13,062.

Report No. 38
 April - June 1972

Environmental Microbiology Section
 Phoenix Laboratories
 Ecological Investigations Program
 Center for Disease Control
 U.S. Department of Health, Education, and Welfare
 Phoenix, Arizona

(NASA-CR-127768) SERVICES PROVIDED IN
 SUPPORT OF THE PLANETARY QUARANTINE
 REQUIREMENTS M.S. Favero (Department of
 Health) Jun. 1972 23 p

N72-29075

CSCL 06M

Unclas

G3/04 38140

Contributors:

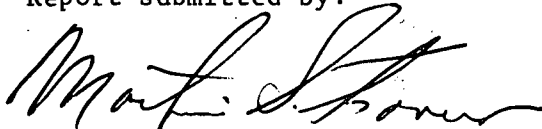
Biophysics Unit Experimental Microbiology Unit Planetary Quarantine Unit

N. Petersen
 J. Marshall
 D. Collins

W. Bond
 L. Carson
 M. Korber

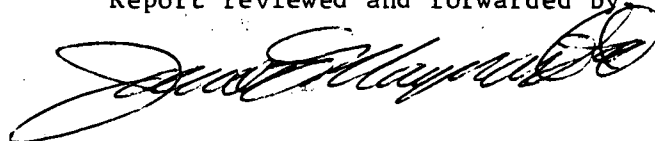
J. Puleo
 G. Oxborrow
 N. Fields
 C. Herring

Report submitted by:



Martin S. Favero, Ph.D., Chief
 Environmental Microbiology Section

Report reviewed and forwarded by:



James E. Maynard, M.D., Chief
 Ecological Investigations Program
 Phoenix Laboratories

1. As reported last quarter, tests were conducted to determine the dry heat resistance at 125 C of a naturally occurring bacterial spore population in a mixture of sieved vacuum cleaner dusts from Cape Kennedy. The dust was aerosolized in a special chamber (Q.R. #37) and was allowed to settle on 32 teflon ribbons (each 3" x 72") to provide approximately 5×10^5 spores per ribbon. Details of the heating and assay procedures are found in Q.R. #37.

Preliminary results (2 weeks of incubation) were reported last quarter. After 28 days of incubation, the results are as follows:

<u>Time at 125C (hr.)</u>	<u>N₀ (spores)</u>	<u>No. +/-</u>	<u>D_{125C} (hr.)</u>	<u>Controls</u>
6	3.7×10^4	2	1.2	0
12	3.8×10^4	2	2.4	0
18	3.4×10^4	0	-	0
24	4.0×10^4	0	-	0

Presence or absence of growth in all jars was confirmed by subculture on TSA and also by placing 1 ml amounts from each jar into tubes of fresh broth. All control jars were negative and the D_{125C} value at each interval was calculated by a method described by Pflug and Schmidt (fractional-replicate-unit-negative-MPN; Q.R. #26).

Five morphologically different survivor isolates were obtained from this test and were identified at the Planetary Quarantine Unit: 6 hr, an actinomycete and Bacillus sphaericus; 12 hr, 2 Bacillus sphaericus and an atypical Bacillus, sp.

In a second trial of the above experiment quite different results were obtained:

<u>Time at 125C (hr.)</u>	<u>N₀ (spores)</u>	<u>No. +/-</u>	<u>D_{125C} (hr.)</u>	<u>Controls</u>
6	5.1×10^4	6	-	0
12	4.1×10^4	4	2.6	0
18	4.4×10^4	4	3.9	0
24	4.6×10^4	2	3.8	0

Growth or absence of growth was confirmed as before, and all control jars were negative. Survivor isolates obtained are in the process of being identified and results will be reported next quarter.

There is no certain explanation for the difference in results between the two experiments. Heating and relative humidity levels during heating were constantly monitored and the arrangement of ribbons in the jars (degree of uncoiling within the jars after being removed from the chamber) were equivalent. The two main differences between the experiments were slightly higher spore levels in the second experiment and the fact that the teflon ribbons in the second experiment were the same (cleaned and re-sterilized) as used in the first experiment. Average spore levels per ribbon (experiment I = 3.7×10^4 ; experiment II = 4.5×10^4) could not alone account for such a great disparity in results. Although the jars negative for growth in the first experiment supported growth of B. subtilis var. niger when inoculated at the end of the 28 day incubation period, the broth used in experiment I may have leached out some component toxic to heat-injured organisms that was not removed by the initial washing and "de-gassing" of new ribbons.

2. At the request of the Planetary Quarantine Officer, the teflon ribbon experiment as described above was conducted at 114 C. An estimated Z-value of 35 C for the naturally occurring spores was assumed and 12-hour heating intervals were used. After 2 weeks incubation, the results are as follows:

<u>Time at 114C (hr.)</u>	<u>N₀ (spores)</u>	<u>No. +/6</u>	<u>D_{114C} (hr.)</u>	<u>Controls</u>
12	4.3×10^4	6	-	0
24	4.1×10^4	6	-	0
36	4.8×10^4	(5)	(8.1)	0
48	4.4×10^4	(2)	(9.5)	0

These preliminary results are from visual observation of turbidity only. Final confirmation of growth by subculture will be made at the end of the 28-day incubation period and results reported next quarter.

3. One gram of the composite dust used in the teflon ribbon experiments above was suspended in 10 ml of filter sterilized 95% ethanol. One-tenth ml amounts were inoculated onto 1/2" x 1/2" stainless steel strips and replicates of 10 strips were heated at each of six 2-hr intervals at 125 C in the usual manner to obtain end-point data for D_{125C} calculation. Survivors were re-covered in the same broth used for the teflon ribbons and tubes were incubated for 28 days:

<u>Time at 125C (hr.)</u>	<u>N₀ (spores)</u>	<u>No. +/10</u>	<u>D_{125C} (hr.)</u>	<u>Controls</u>
0	2.9×10^2	-	-	0
2	2.9×10^2	10	-	0

(Table continued on page 3)

<u>Time at 125C (hr.)</u>	<u>N_O (spores)</u>	<u>No. +/-10</u>	<u>D_{125C} (hr.)</u>	<u>Controls</u>
4	2.9 x 10 ²	3	1.4	0
6	2.9 x 10 ²	0	-	0
8	2.9 x 10 ²	0	-	0
10	2.9 x 10 ²	0	-	0
12	2.9 x 10 ²	0	-	0

4. To conclude heat resistance testing of bacterial spores at the Phoenix Laboratories, the D_{114C} of the standard B. subtilis var. niger spore crop (SSM-10 liquid medium) will be determined. Results will be reported next quarter in addition to a compilation of D_{125C} values (FN-MPN data) obtained with spore crops of a variety of isolates from biodegradation grinder assays, heat tests of dusts and teflon ribbons, and hospital surveys in the Phoenix area.

The highly dry-heat resistant bacterial spore isolated from Cape Kennedy soil (Q.R. #33-36) was submitted to the American Type Culture Collection and has an accession number of 27380.

5. It is commonly assumed that the slit air sampler impacts airborne viable particles onto an agar surface without significantly altering the distribution of particle sizes and consequently clump sizes occurring naturally in the air. This assumption was questioned after extensive tests showed that levels detected by membrane filters consistently were less than those detected by a slit sampler. (Q.R. #36) One explanation for this finding suggested that large particles passing through the sampler slit were fragmented into several smaller particles resulting in an over-estimation of the number of airborne viable particles. To explore this possibility, tests were performed on the total airborne particulate population using a Royco particle counting and sizing instrument. The instrument and laboratory space for this experiment were provided by the Planetary Quarantine Program of Sandia Laboratories. The particle size distribution in 30 samples of ambient air was compared with the particle size distribution in 30 samples of ambient air which had passed through the slit of a Reyniers sampler. These distributions are presented in Table 1 and show that air passed through a slit has a significantly higher percentage of particles in the .05-.08 micron range and significantly lower percentages in the 3-6 and 6-25 micron ranges than ambient air. This tends to support the theory of large particles breaking into smaller particles as a result of passing through a slit.

The theory was tested for viable particles by fitting a Reyniers slit to the top of an Andersen sampler and sampling with this sampler and a standard Andersen sampler at contiguous sites in a room with well mixed air.

The particle size distributions for 10 paired samples are presented in Table 2. The results are in agreement with those for the total particulate distribution in that air which has passed through a slit has a significantly lower percent of large particles and a significantly higher percent of small particles than does ambient air.

These results suggest that large viable airborne particles do break into smaller viable particles when sampled in a slit sampler. However, one reservation in arriving at this conclusion has been the finding that when a slit is fitted to an Andersen sampler the total number of viable particles collected is approximately 50% of the total number collected in a standard Andersen sampler. When a Reyniers slit sampler is compared with a standard Andersen sampler no significant difference is seen in the total number of viable particles collected. Therefore, it is not the slit, per se, that reduces the total number of particles collected. Until a system can be devised that will result in comparable total numbers of viable particles in both the standard Andersen and the slit equipped Andersen, it cannot be concluded that the differences observed in particle size distributions were due to fragmentation of large viable particles rather than a simple unexplained loss of large viable particles.

6. Additional tests have been conducted to characterize the efficacy of the wipe-rinse technique in sampling large surfaces contaminated with naturally occurring microorganisms. The details of the procedures used in these experiments have been described previously (Q.R. #37). A total of 20 aluminum trays, each with an exposed surface of 168 in² were sampled with moistened "Handi-Wipes." The mean level of contamination on these trays was estimated to be 68 microorganisms per 4 in² based on standard assays of forty 2" x 2" stainless steel strips. The mean recovery value for these 20 wipe-rinse assays was 44% with a coefficient of variation of 48%.

The experimental design was then changed to use a larger aluminum surface having a lower level of naturally occurring contamination. Wipe-rinse samples were taken from 28 surfaces each with an exposed area of 1280 in² (equivalent to 320 swab-rinse areas). The mean level on these surfaces was estimated to be 29 microorganisms per 4 in² based on the assays of sixty-eight 2" x 2" stainless steel strips. The mean recovery value for the wipe-rinse assays of these surfaces was 47% with a coefficient of variation of 40%. Half of these surfaces were wiped with dry cloths and half with moistened cloths. The mean recovery value for moistened cloths was slightly higher than that for wet cloths (51% vs. 43%); however, this difference was not statistically significant.

These tests have demonstrated that the wipe-rinse technique has a mean recovery value of 46% when used on large, smooth surfaces. The comparable value for the standard swab technique in the hands of experienced individuals was found to be 43% (Q.R. #32). While the overall coefficient of variation for the wipe-rinse technique was 43% the value for the swab-rinse technique was 31%. However, this advantage of smaller variation in swab-rinse recovery is heavily outweighed by the fact that the wipe-rinse

technique is capable of measuring the total population of microorganisms on surfaces rather than a sample constituting only a fraction of the population. The time required to wipe a surface several hundred times as large as the 4 in² sample used in the swab-rinse technique is only slightly greater than that required for taking a single swab-rinse sample. The time required for assay of a wipe-rinse sample is identical to that required for a swab-rinse sample.

7. An evaluation to determine whether the M/G slit air sampler was equivalent to the Reyniers slit sampler was continued. Results of tests conducted on the first model were reported previously (Q.R. #37) and demonstrated that inadequate pump size resulted in significantly lower counts in samples collected with the M/G sampler than in samples collected with the Reyniers. In discussions with the manufacturer, it was agreed that a larger pump was required and a second model with a larger pump was provided for evaluation. This model used the same type flow rate gauge as the first model and correction factors necessary to convert gauge readings to true flow rates through the slit were not provided. Accordingly, to achieve the desired sampling rate of 60 cfh (1 cfm) it was necessary to operate the sampler at an indicated flow rate of 70 cfh. This, incidentally, was the maximum flow rate the larger pump was able to achieve and consequently provided no margin for pump wear or "fading" due to heating of the electric motor. Tests showed that as the motor temperature rose the flow rate decreased, stabilizing at 55 cfh after approximately 60 minutes of operation.

The sampling capability of the M/G sampler was compared with that of two Reyniers samplers by operating all three samplers simultaneously at contiguous sites in a room with well mixed air for a total of twenty-five 30-minute sampling periods. The mean concentrations detected by the two Reyniers samplers were 2.7 and 3.0 viable particles per ft³ respectively while the mean concentration detected by the M/G sampler was 2.7 particles per ft³. Statistical tests showed that the observed differences were not significant. Levels detected by the Reyniers samplers also were compared with those levels detected by the M/G sampler when operating under reduced flow rate, i.e. hot pump motor conditions. The values were 2.3 and 2.4 viable particles per ft³ for the M/G and Reyniers respectively. This difference was not significant. A non-parametric statistical test (sign test) further demonstrated that the number of times the M/G sampler detected a lower level than a Reyniers sampler during the same sampling period was not statistically significant.

Based on the results of this evaluation it appears that the M/G sampler with the large pump is equivalent to the Reyniers sampler provided the M/G is operated at an indicated flow rate of 70 cfh.

8. Microbiological studies were performed on the Apollo 16 spacecraft during assembly and testing at Kennedy Space Center (KSC). Swab samples were taken on the interior surfaces of the Command Module (CM) at pre-flight (T-9 hours) at KSC and post-flight on the recovery vessel. Pre-flight samples were taken by a member of the astronaut backup crew and the post-

flight sample was taken by a staff member of the Manned Space Center (MSC) present on the recovery vessel.

Table 3 shows the results of the individual surface sites sampled on pre- and post-flight. The microbial samples taken on post-flight were not treated according to the protocol agreement between MSC and this laboratory. Specifically, the following portions of the post-flight protocol were violated:

1. Microbial samples were not placed into screw cap test tubes, but placed in snap-on plastic vials which were placed into a plastic jar container. This resulted in the liquid menstrum leaking from the snap-on plastic vials.
2. Samples were not maintained at 4 C during transport to Cape Kennedy, Florida. The container holding the samples was found to be floating in water upon arrival in Houston. Prior to shipment to Cape Kennedy, ice was added.

These deviations resulted in obtaining sample tubes which had leaked and contained varying amounts of broth in each tube. The thawing and cooling of the samples could have resulted in loss of cell viability. Possibility of contamination due to leaking tubes also must be considered. Furthermore, post-flight samples taken on Apollo 15 were delivered to Cape Kennedy within 30 hours after being collected. Samples from Apollo 16 were delivered to Cape Kennedy 56 hours after collection. Due to the unsatisfactory conditions in which the post-flight samples were treated prior to being received at Cape Kennedy for assay, the quantitative and qualitative results were not considered reliable. Accordingly, no valid comparison can be made with pre-flight samples. Action has been initiated to preclude such a recurrence on future missions.

Average levels of microbial contamination on the pre-flight samples, with few exceptions, were similar to those detected on the CM of Apollo 15 when identical sites were compared (Table 4). The levels of microorganisms encountered on the pre-flight sample of the drink gun were similar to those found on the post-flight sample of the drink gun of Apollo 15 (Q.R. #35). For purpose of comparison, the fifteen sampling sites in the CM were combined, and the mean number of aerobic mesophilic microorganisms per square foot was determined for pre-flight samples. This number, 6.1×10^4 per square foot, compares well with results obtained from previous Apollo CM (Q.R. #35).

A total of 390 microorganisms were isolated and identified from the CM of Apollo 16. Of these isolates, 245 were obtained from the interior surfaces at pre-flight. Table 5 shows the number and types of microorganisms detected on pre- and post-flight from all media and incubation conditions employed.

The majority (97.1%) of microorganisms detected on pre-flight samples were types considered indigenous to human hair, skin, and respiratory tract.

The occurrence of microorganisms associated with soil and dust in the environment were low. These results are comparable to those obtained from previous CM (Q.R. #36).

The types of microorganisms detected on pre- and post-flight on Trypticase Soy Agar (TSA) and Blood Agar (BA) are listed in Tables 6 and 7. A total of 18 types or groups of microorganisms were identified on pre-flight (Table 6) and 12 on post-flight samples (Table 7). Pre-flight isolates also includes those microorganisms isolated from samples which were heat-shocked. Eosin-Methylene-Blue Agar (EMB) was used in an effort to enhance the recovery of gram-negative microorganisms, particularly on the post-flight samples. Gram-negative microorganisms were not recovered from either pre- or post-flight samples and no yeasts or molds were detected.

Table 8 shows a summary of all the types of microorganisms detected on the CM of Apollo spacecraft 10 thru 16 prior to launch. This table includes only those mesophilic microorganisms isolated from samples not exposed to heat shock and grown aerobically on TSA. The prevalent types found were Staphylococcus sp. (Subgroups II thru VI), Micrococcus sp. (Subgroups 1, 2, 3, 5, and 7), Corynebacterium-Brevibacterium Group, and yeasts.

The microorganisms isolated and identified from the CM of Apollo 16 were lyophilized and stored for future reference. In addition, all data pertaining to enumeration and identification of microorganisms from the Apollo spacecraft were treated and stored on a CDC 3600 computer at Cape Kennedy for rapid retrieval. Computer printouts were compiled and sent to the Planetary Quarantine Officer.

9. The study to determine the thermal resistance of naturally occurring airborne spores (Q.R. #35-37) was continued in the "new area" (Q.R. #37) using the heating times 2, 4, 6, and 8 hours. Thus far, twenty-six (26) experiments have been run in the "new area." Table 9 summarizes the data for experiments 17 to 30. The N_0 values shown are the average of eight control strips per experiment.

A summary of all ribbon experiments to date according to heating time and location is shown in Table 10. The N_0 values shown are the average of all control strips at that heating interval (two per experiment). Survivors have been recovered at the longest heating time of 8 hours. D_{125C} values were calculated using the FN-MPN technique of Pflug and Schmidt. D_{125C} values ranged from 25 to 130 minutes depending on the length of exposure to heat.

TABLE 1. COMPARISON OF PARTICLE SIZE DISTRIBUTION IN AMBIENT AIR WITH THE DISTRIBUTION IN AMBIENT AIR PASSED THROUGH A SLIT

Particle Size <u>Microns</u>	<u>% of Particles in Range</u>		<u>t</u>
	<u>Ambient Air</u>	<u>Ambient Air Through Slit</u>	
0.5-0.8	50.43	51.85	3.8243*
0.8-2.0	28.69	28.71	0.1817
2.0-3.0	15.25	15.01	1.0803
3.0-6.0	4.73	3.85	5.7414*
6.0-25	.90	.57	7.4903*

* Significant $P < .001$

TABLE 2. COMPARISON OF VIABLE PARTICLE SIZE DISTRIBUTION IN AMBIENT AIR
WITH THE DISTRIBUTION IN AMBIENT AIR PASSED THROUGH A SLIT

<u>Particle Size Microns</u>	<u>% of Particles in Range</u>		<u>t</u>
	<u>Ambient Air</u>	<u>Ambient Air Through Slit</u>	
<1.0	0.4	0.5	0.3181
1.0-2.0	24.1	39.7	3.8906**
2.0-3.3	16.6	22.3	3.4304**
3.3-5.5	14.7	14.5	0.0555
5.5-9.2	15.6	6.7	4.8546*
>9.2	28.6	16.5	3.3348**

* Significant $P < .001$

** Significant $P < .01$

TABLE 3. COMPARISON OF THE PRE AND POST FLIGHT MICROBIOLOGICAL RESULTS OF THE INDIVIDUAL SURFACE SITES SAMPLED IN APOLLO 16 COMMAND MODULE

<u>Areas Sampled</u>	<u>Mean No. Microorganisms Per Square Inch</u>	
	<u>T-9 Hrs.</u>	<u>Post-Flight</u>
Girth Shelf - Right	2.5×10^2	4.5×10^1
Girth Shelf - Left	8.5×10^2	2.9×10^2
Waste Disposal Rim (Compartment No. 5)	9.8×10^1	5.3×10^2
Top Flight Recorder (Flight Tape Recorder)	1.5×10^2	4.8×10^1
Reaction Jet Control (On-Off)	8.6×10^1	7.5×10^0
Exposed Floor by Hatch	2.4×10^2	2.0×10^1
Ordeal Cable Stowage (Top)	6.2×10^2	0.0
Vertical Couch Support Beam - Right	8.7×10^0	1.0×10^2
Vertical Couch Support Beam - Left	8.7×10^0	1.2×10^2
Horizontal Couch Support Beam - Right	1.4×10^2	3.5×10^1
Horizontal Couch Support Beam - Left	2.4×10^3	8.6×10^2
Ledge Below Left Window	9.8×10^2	2.5×10^0
Right Control Handle (RHC)	3.3×10^1	0.0
Left Control Handle (LHC)	3.8×10^1	2.5×10^0
Drink Gun ¹	1.5×10^3	2.7×10^2

¹ Total number of microorganisms recovered from sample.

TABLE 4. COMPARISON OF THE PRE FLIGHT MICROBIOLOGICAL RESULTS OF THE INDIVIDUAL SURFACE SITES SAMPLED IN APOLLO 15 AND 16 COMMAND MODULE

<u>Areas Sampled</u>	<u>Mean No. Microorganisms Per Square Inch¹</u>	
	<u>Apollo 15</u>	<u>Apollo 16</u>
Girth Shelf - Right	7.5×10^1	2.5×10^2
Girth Shelf - Left	1.3×10^2	8.5×10^2
Waste Disposal Rim (Compartment No. 5)	8.8×10^1	9.8×10^1
Top Flight Recorder (Flight Tape Recorder)	1.3×10^2	1.5×10^2
Reaction Jet Control (On-Off)	5.0×10^1	8.6×10^1
Exposed Floor by Hatch	1.5×10^2	2.4×10^2
Ordeal Cable Stowage (Top)	5.8×10^1	6.2×10^2
Vertical Couch Support Beam - Right	2.5×10^0	8.7×10^0
Vertical Couch Support Beam - Left	0.0	8.7×10^0
Horizontal Couch Support Beam - Right	3.3×10^2	1.4×10^2
Horizontal Couch Support Beam - Left	1.5×10^2	2.4×10^3
Ledge Below Left Window	2.3×10^1	9.8×10^2
Right Control Handle (RHC)	0.0	3.3×10^1
Left Control Handle (LHC)	0.0	3.8×10^1
Drink Gun ²	0.0	1.5×10^3

¹ Aerobic mesophilic count.

² Total number of microorganisms recovered from sample.

TABLE 5. COMPARISON OF THE TYPES OF MICROORGANISMS DETECTED FROM THE
COMMAND MODULE OF APOLLO 16

	Pre-Flight (T-9 Hours)	Post-Flight
<u>Staphylococcus</u> spp.		
Subgroup II	+	+
Subgroup III	+	+
Subgroup IV	+	+
Subgroup V	+	+
Subgroup VI	+	+
<u>Micrococcus</u> spp.		
Subgroup 1	+	+
Subgroup 2	+	+
Subgroup 3	+	-
Subgroup 7	+	+
<u>Bacillus</u> spp.		
<u>B. badius</u>	+	-
<u>B. circulans</u>	+	+
<u>B. firmus</u>	+	-
<u>B. lentus</u>	+	-
<u>B. polymyxa</u>	+	+
<u>B. subtilis</u>	+	-
<u>Corynebacterium-Brevibacterium</u> Group		
	+	+
Atypical <u>Micrococcus</u> spp.	+	+
Atypical <u>Bacillus</u> spp.	+	-
Number Isolated		
	245	145

TABLE 6. TYPES OF MICROORGANISMS DETECTED ON PRE-FLIGHT (T-9 HOURS) FROM THE COMMAND MODULE OF APOLLO 16 ON VARIOUS MEDIA

	INCUBATION CONDITIONS			
	Aerobic		Anaerobic	
	TSA	BA	TSA	BA
<u>Staphylococcus</u> spp.				
Subgroup II	+	+	+	+
Subgroup III	+	+	+	-
Subgroup IV	+	+	+	-
Subgroup V	+	+	+	+
Subgroup VI	+	+	+	+
<u>Micrococcus</u> spp.				
Subgroup 1	+	-	-	-
Subgroup 2	+	-	-	-
Subgroup 3	+	+	-	-
Subgroup 7	+	+	+	+
<u>Bacillus</u> spp.				
<u>B. badius</u>	+	-	-	-
<u>B. circulans</u>	+	-	+	-
<u>B. firmus</u>	+	-	-	-
<u>B. lentus</u>	+	-	-	-
<u>B. polymyxa</u>	+	-	+	-
<u>B. subtilis</u>	+	-	-	-
<u>Corynebacterium-Brevibacterium</u>				
Group	+	+	+	+
Atypical <u>Micrococcus</u> spp.	+	-	+	+
Atypical <u>Bacillus</u> spp.	+	-	+	-
Number Isolated				
	148	17	65	15

TSA - Trypticase Soy Agar
BA - Blood Agar

TABLE 7. TYPES OF MICROORGANISMS DETECTED ON POST-FLIGHT FROM THE
COMMAND MODULE OF APOLLO 16 ON VARIOUS MEDIA

	INCUBATION CONDITIONS			
	Aerobic		Anaerobic	
	TSA	BA	TSA	BA
<u>Staphylococcus</u> spp.				
Subgroup II	+	+	+	+
Subgroup III	+	-	-	-
Subgroup IV	+	-	+	+
Subgroup V	+	+	+	+
Subgroup VI	-	-	+	-
<u>Micrococcus</u> spp.				
Subgroup 1	+	+	-	-
Subgroup 2	+	-	-	-
Subgroup 7	+	-	+	-
<u>Bacillus</u> spp.				
<u>B. circulans</u>	-	-	+	-
<u>B. polymyxa</u>	-	+	-	-
<u>Corynebacterium-Brevibacterium</u>				
Group	+	+	-	-
Atypical <u>Micrococcus</u> spp.	+	-	-	-
Number Isolated	82	10	47	6

TSA - Trypticase Soy Agar
BA - Blood Agar

TABLE 8. COMPARISON OF THE TYPES OF MICROORGANISMS DETECTED ON TRYPTICASE SOY AGAR FROM COMMAND MODULES OF APOLLO 10 THRU 16

TYPE OF MICROORGANISM ¹	MISSION						
	10	11	12	13	14	15	16
<u>Staphylococcus</u> spp.							
Subgroup I	-	+	-	-	-	+	-
Subgroup II	+	+	+	+	+	+	+
Subgroup III	+	+	+	-	+	+	+
Subgroup IV	+	+	+	+	+	+	+
Subgroup V	+	+	+	+	+	+	+
Subgroup VI	+	+	+	+	+	+	+
<u>Micrococcus</u> spp.							
Subgroup 1	+	+	+	+	+	+	+
Subgroup 2	+	+	+	+	+	+	+
Subgroup 3	+	+	+	+	-	+	+
Subgroup 4	-	-	-	-	-	+	-
Subgroup 5	+	+	+	-	+	+	-
Subgroup 6	-	-	-	-	-	-	-
Subgroup 7	+	+	+	+	+	+	+
<u>Streptococcus-Viridans</u>							
Group	-	+	-	-	-	-	-
<u>Bacillus</u> spp.							
<u>B. badius</u>	-	-	-	-	-	+	+
<u>B. brevis</u>	+	-	-	-	-	-	-
<u>B. cereus</u>	+	-	+	-	-	+	-
<u>B. circulans</u>	+	-	-	-	+	+	+
<u>B. coagulans</u>	-	-	-	-	+	+	-
<u>B. firmus</u>	-	-	+	-	+	-	-
<u>B. lentus</u>	+	-	-	-	-	-	-
<u>B. licheniformis</u>	-	+	-	-	-	+	-
<u>B. macerans</u>	-	-	-	-	+	-	-
<u>B. pantothenicus</u>	-	-	+	-	-	-	-
<u>B. polymyxa</u>	+	+	-	-	+	+	-
<u>B. pulvifaciens</u>	+	-	+	-	-	-	-
<u>B. pumilus</u>	-	-	-	-	+	-	-
<u>B. sphaericus</u>	-	-	+	-	-	-	-
<u>B. subtilis</u>	-	-	-	+	-	-	-

TABLE 8. COMPARISON OF THE TYPES OF MICROORGANISMS DETECTED ON TRYPTICASE SOY AGAR FROM COMMAND MODULES OF APOLLO 10 THRU 16 - (Continued)

TYPE OF MICROORGANISM ¹	MISSION						
	10	11	12	13	14	15	16
<u>Corynebacterium-Brevibacterium</u> Group	+	+	+	+	+	+	+
<u>Alcaligenes</u> spp.	-	-	+	-	-	-	-
<u>Flavobacterium</u> spp.	+	-	-	-	-	-	-
Yeasts	+	-	+	+	-	+	-
Molds	+	-	-	-	-	-	-
Atypical <u>Micrococcus</u> spp.	-	+	+	+	+	+	+
Atypical <u>Bacillus</u> spp.	-	-	-	-	+	+	+
Number Isolated	285	300	290	256	204	250	139

¹ Aerobic, mesophilic microorganisms isolated from non-heat shocked samples.

TABLE 9. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS -
CAPE KENNEDY

EXPERIMENT NUMBER	TOTAL COUNT	No		MOLDS	SURVIVORS	IDENTIFICATION
			SPORES			
1	Old Area	5.4x10 ³	2.4x10 ²	2.4x10 ²	3 hr. - 3/6 6 hr. - 0/6 9 hr. - 0/6 12 hr. - 0/6	<u>B. circulans-2</u> , Atypical <u>Bacillus</u>
2	"	4.0x10 ³	1.2x10 ²	8.0x10 ¹	3 hr. - 0/6 6 hr. - 0/6 9 hr. - 0/6 12 hr. - 0/6	
3	"	3.1x10 ³	1.4x10 ²	1.6x10 ²	3 hr. - 0/6 6 hr. - 0/6 9 hr. - 0/6 12 hr. - 0/6	
4	"	4.1x10 ³	4.3x10 ²	1.6x10 ²	3 hr. - 0/6 6 hr. - 1/6 9 hr. - 0/6 12 hr. - 0/6	Atypical <u>Bacillus</u>
5	New Area	4.9x10 ³	2.7x10 ²	3.6x10 ³	1 hr. - 5/6 2 hr. - 3/6 3 hr. - 1/6 4 hr. - 1/6	<u>B. sphaericus-2</u> , <u>B. lentus</u> , <u>B. firmus</u> , Atypical <u>Bacillus</u> <u>B. cereus</u> , <u>B. firmus</u> , Atypical <u>Bacillus</u> <u>B. polymyxa</u> <u>B. subtilis</u>
6	"	3.8x10 ³	2.6x10 ²	2.6x10 ³	1 hr. - 4/6 2 hr. - 3/6 3 hr. - 0/6 4 hr. - 2/6	<u>B. lentus-2</u> , <u>B. subtilis</u> , Atypical <u>Bacillus</u> <u>B. polymyxa</u> , <u>B. pantotheneticus</u> , Atypical <u>Bacillus</u> <u>B. lentus-2</u>

TABLE 9. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS -
CAPE KENNEDY (Continued)

EXPERIMENT NUMBER	New Area	No		MOLDS	SURVIVORS	IDENTIFICATION
		TOTAL COUNT	SPORES			
7	New Area	1.4x10 ³	1.4x10 ²	4.8x10 ²	1 hr. - 1/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	<u>B. sphaericus</u>
8	"	4.0x10 ²	4.0x10 ¹	2.4x10 ²	1 hr. - 0/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	
9	"	4.8x10 ²	5.6x10 ¹	8.0x10 ¹	1 hr. - 0/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	
10	"	5.6x10 ²	1.5x10 ²	1.6x10 ²	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
11	"	1.3x10 ³	2.1x10 ²	4.0x10 ²	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
12	"	6.4x10 ²	8.8x10 ¹	2.4x10 ²	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
13	"	1.4x10 ³	1.4x10 ²	9.6x10 ²	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	

TABLE 9. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS -
CAPE KENNEDY (Continued)

EXPERIMENT NUMBER	New Area	$\frac{N_0}{\text{TOTAL COUNT}}$		MOLDS	SURVIVORS	IDENTIFICATION
		TOTAL COUNT	SPORES			
14	New Area	1.1×10^3	1.3×10^2	2.4×10^2	2 hr. - 3/6 4 hr. - 1/6 6 hr. - 0/6 8 hr. - 0/6	<u>B. circulans</u> , Actinomycete, Atypical <u>Bacillus</u> Actinomycete
15	"	4.0×10^2	5.6×10^1	1.6×10^2	2 hr. - 1/6 4 hr. - 1/6 6 hr. - 0/6 8 hr. - 0/6	<u>B. sphaericus</u> <u>B. sphaericus</u>
16	"	4.0×10^2	7.2×10^1	1.6×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
17	"	6.4×10^2	6.4×10^1	3.2×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
18	"	1.5×10^3	2.1×10^2	4.0×10^2	2 hr. - 1/6 4 hr. - 0/6 6 hr. - 2/6 8 hr. - 0/6	<u>B. sphaericus</u> <u>B. circulans</u> , Atypical <u>Bacillus</u>
19	"	4.8×10^2	4.8×10^1	8.0×10^1	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	

TABLE 9. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS -
CAPE KENNEDY (Continued)

EXPERIMENT NUMBER	No	TOTAL COUNT		SPORES	MOLDS	SURVIVORS	IDENTIFICATION
20	New Area	1.9x10 ³	2.2x10 ²	4.0x10 ²	2 hr. - 2/6 4 hr. - 0/6 6 hr. - 1/6 8 hr. - 1/6	Actinomycete, In Process <u>B. lentus</u> <u>B. sphaericus</u>	
21	"	2.4x10 ²	5.6x10 ¹	8.0x10 ¹	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6		
22	"	5.6x10 ²	8.0x10 ¹	8.0x10 ¹	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6		
23	"	7.2x10 ²	8.0x10 ¹	2.4x10 ²	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6		
24	"	9.6x10 ²	1.1x10 ²	2.4x10 ²	2 hr. - 1/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	<u>B. lentus</u>	
25	"	2.9x10 ³	5.2x10 ²	8.8x10 ²	2 hr. - 1/6 4 hr. - 1/6 6 hr. - 0/6 8 hr. - 0/6	<u>B. sphaericus</u> <u>B. sphaericus</u>	
26	"	2.5x10 ³	8.2x10 ²	3.2x10 ²	2 hr. - 3/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 3/6	<u>B. subtilis</u> , <u>B. lentus</u> -2 <u>B. sphaericus</u> -2, In Process	

TABLE 9. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS -
CAPE KENNEDY (Continued)

EXPERIMENT NUMBER		TOTAL COUNT	$\frac{N_0}{\text{SPORES}}$	MOLDS	SURVIVORS	IDENTIFICATION
27	New Area	2.0×10^3	6.6×10^2	4.0×10^2	2 hr. - 1/6 4 hr. - 1/6 6 hr. - 0/6 8 hr. - 1/6	Lost in Process Lost in Process Actinomycete
*28	"	3.2×10^3	2.3×10^2	3.2×10^2	2 hr. - 3/6 4 hr. - 0/6 6 hr. - 1/6 8 hr. - 0/6	In Process In Process
*29	"	1.8×10^3	2.1×10^2	7.2×10^2	2 hr. - 1/6 4 hr. - 1/6 6 hr. - 0/6 8 hr. - 0/6	In Process In Process
*30	"	1.0×10^3	1.7×10^2	1.6×10^2	2 hr. - 1/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	In Process

* Experiments still incubating.

TABLE 10. SUMMATION OF RESULTS FROM TEFLON RIBBON EXPERIMENTS - CAPE KENNEDY

MSOB - NEW AREA

HEATING TIME	NO. OF EXPERIMENTS	VIABLE	NO SPORES	MOLDS	NO. SURVIVORS PER TOTAL NO. FLASKS	D _{125C} VALUE	IDENTIFICATION
1 hr.	5	2.7x10 ³	1.0x10 ²	1.8x10 ³	10/30	25 min.	<u>B. lentus</u> -3, <u>B. sphaericus</u> -3, <u>B. firmus</u> , <u>B. subtilis</u> , Atypical <u>Bacillus</u> -2
2 hr.	26	1.4x10 ³	1.9x10 ²	4.8x10 ²	25/156*	53 min.	<u>B. sphaericus</u> -3, <u>B. cereus</u> , <u>B. firmus</u> , <u>B. polymyxa</u> , <u>B. subtilis</u> , <u>B. pantothenicus</u> , <u>B. lentus</u> -4, <u>B. circulans</u> , Atypical <u>Bacillus</u> -2, Actinomycete-3, Lost in Process-2, In Process-5
3 hr.	5	1.8x10 ³	2.1x10 ²	1.2x10 ³	1/30	47 min.	<u>B. polymyxa</u>
4 hr.	26	1.4x10 ³	2.1x10 ²	5.6x10 ²	9/156*	68 min.	<u>B. subtilis</u> , <u>B. lentus</u> -2, <u>B. sphaericus</u> -2, Actinomycete, In Process-2, Lost in Process
6 hr.	21	1.2x10 ³	2.0x10 ²	2.4x10 ²	4/126*	95 min.	In Process, <u>B. circulans</u> , <u>B. lentus</u> , Atypical <u>Bacillus</u>
8 hr.	21	1.4x10 ³	2.0x10 ²	3.2x10 ²	5/126*	130 min.	<u>B. sphaericus</u> -3, Actinomycete, Lost in Process

MSOB - OLD AREA

3 hr.	4	3.6x10 ³	2.2x10 ²	8.0x10 ¹	3/24	56 min.	<u>B. circulans</u> -2, Atypical <u>Bacillus</u>
6 hr.	4	4.3x10 ³	2.2x10 ²	1.6x10 ²	1/24	97 min.	Atypical <u>Bacillus</u>
9 hr.	4	4.0x10 ³	2.0x10 ²	1.6x10 ²	0/24	--	----
12 hr.	4	4.6x10 ³	2.6x10 ²	2.4x10 ²	0/24	--	----

* Includes experiments still incubating.